Hubbard, S. C., & Ivatt, R. J. (1981) *Annu. Rev. Biochem. 50*, 555–583.
Hui, S. W., Stewart, T. P., Yeagle, P. L., & Albert, A. D. (1981) *Arch. Biochem. Biophys. 207*, 227–240.
Jensen, J. W., & Schutzbach, J. S. (1981) *J. Biol. Chem. 256*, 12899–12904.
Jensen, J. W., & Schutzbach, J. S. (1982) *J. Biol. Chem. 257*, 9025–9029.
Jensen, J. W., Springfield, J. D., & Schutzbach, J. S. (1980) *J. Biol. Chem. 255*, 11268–11272.
Madden, T. D., & Cullis, P. R. (1982) *Biochim. Biophys. Acta 684*, 149–153.
Schutzbach, J. S., Springfield, J. D., & Jensen, J. W. (1980) *J. Biol. Chem. 255*, 4170–4175.
Stollery, J. G., & Vail, W. J. (1977) *Biochim. Biophys. Acta 471*, 372–390.
Struck, D. K., & Lennarz, W. J. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., Ed.) pp 35–83, Plenum Press, New York.
Szoka, F., Jr., & Papahadjopoulos, D. (1980) *Annu. Rev. Biophys. Bioeng. 9*, 467–508.
Tilcock, C. P. S., & Cullis, P. R. (1982) *Biochim. Biophys. Acta 684*, 212–218.
Tilcock, C. P. S., Bally, M. B., Farren, S. B., & Cullis, P. R. (1982) *Biochemistry 21*, 4596–4601.

Thermal Behavior of Cores of Human Serum Triglyceride-Rich Lipoproteins: A Study of Induced Circular Dichroism of β-Carotene†

G. Chi Chen, John P. Kane,* and Robert L. Hamilton

ABSTRACT: Induced circular dichroism (CD) of β-carotene has been used to study the physical state in the cores of three classes of triglyceride-rich lipoproteins from human serum: intermediate-density lipoproteins (IDL) ($1.006 < d < 1.019$ g/mL) and subfractions of the $d < 1.006$ g/mL lipoproteins of β and pre-β electrophoretic mobility. Effects on the physical state in the cores attributable to the ratio of triglycerides to cholesteryl esters and particle diameters were assessed by comparing the temperature-dependent CD spectra of β-carotene with those of low-density lipoproteins (LDL). Lipoproteins were prepared from serum by sequential ultracentrifugation after the donors were given supplemental dietary β-carotene (60 mg/day) for 2 weeks. The β- and pre-β-migrating $d < 1.006$ g/mL lipoproteins were separated by starch block electrophoresis and were then individually separated into subfractions by agarose gel filtration chromatography. Between 7 and 30 °C, four subfractions of the β-migrating $d <$ 1.006 g/mL lipoproteins and IDL exhibited reversible, temperature-dependent induced CD of β-carotene, with contours similar to those of LDL but with smaller magnitudes and much broader transitions of the CD bands than those of LDL. In contrast, subfractions of the pre-β-migrating $d < 1.006$ g/mL lipoproteins showed no detectable induced CD of β-carotene. These results show that the cores of triglyceride-rich lipoproteins can exist in some ordered state between 7 and 30 °C if they have a relatively low ratio of triglycerides to cholesteryl esters (mass ratio <1.6) and relatively small particle diameter (<60 nm).

The cores of human serum very low density lipoproteins (VLDL)[1] are enriched in triglycerides, whereas those of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) chiefly contain cholesteryl esters (Skipski, 1972). LDL undergo a reversible thermal transition around body temperature, reflecting a change in the physical state of their core cholesteryl esters from an ordered liquid-crystalline phase to a disordered liquid phase, as shown by studies of differential scanning calorimetry, X-ray scattering, polarizing microscopy, nuclear magnetic resonance spectroscopy, and a β-carotene circular dichroism (CD) probe technique (Deckelbaum et al., 1975, 1977a; Sears et al., 1976; Atkinson et al., 1977; Laggner et al., 1977; Kroon, 1981; Chen & Kane, 1974). Unlike LDL, normal human VLDL and HDL do not undergo similar thermal changes in the physical state of their core cholesteryl esters (Deckelbaum et al., 1977b; Tall et al., 1977b).

A change in the physical state of the cores in lipoproteins is greatly influenced by the composition of the core lipids and by the diameter of the lipoprotein particles (Deckelbaum et al., 1977a; Laggner et al., 1977; Tall et al., 1977b; Tall, 1980). The transition temperature depends on the ratio of triglycerides to cholesteryl esters and on the degree of saturation of cholesteryl ester fatty acids (Deckelbaum et al., 1977a; Pownall et al., 1980a,b; Tall et al., 1977a, 1978; Kirchhausen et al., 1979; Kroon & Seidenberg, 1982). Thus, the absence of a transition in normal human VLDL has been attributed to their high content of triglycerides, indicating that the core cholesteryl esters are dissolved in the liquid triglycerides (Deckelbaum et al., 1977b). Furthermore, studies of the physical state of the cores in cholesteryl ester rich lipoproteins from various species have shown that their core cholesteryl esters can undergo an ordered/disordered state transition if the particle diameter is greater than 14 nm (Deckelbaum et al., 1977a; Laggner et al., 1977; Tall, 1980). Thus, the absence of a transition in human HDL has been attributed to their diameter, which is too small to accommodate the formation of an ordered state of cholesteryl esters in the cores (Tall et al., 1977b; Tall, 1980), or the domain of cholesteryl esters in HDL is too small to exhibit cooperative melting. It is not yet known whether the core cholesteryl esters in some triglyceride-rich

† From the Cardiovascular Research Institute and the Departments of Medicine and Anatomy, University of California, San Francisco, California 94143. *Received July 5, 1983.* This work was supported by Arteriosclerosis SCOR Grant HL 14237 from the U.S. Public Health Service.

* Correspondence should be addressed to this author at the Cardiovascular Research Institute, University of California.

[1] Abbreviations: VLDL, very low density lipoproteins; HDL, high-density lipoproteins; LDL, low-density lipoproteins; IDL, intermediate-density lipoproteins; CD, circular dichroism; EDTA, ethylenediaminetetraacetic acid.

0006-2960/84/0423-1119$01.50/0

lipoproteins exist in any ordered state or separate domains and how low a ratio of triglycerides to cholesteryl esters and how small a particle diameter are necessary for the occurrence of changes in the physical state of their cores. To answer these questions, we studied two classes of triglyceride-rich lipoproteins whose ratios of triglycerides to cholesteryl esters and whose particle diameters are between those of LDL and VLDL: the intermediate-density lipoproteins (IDL) and the subfractions of $d < 1.006$ g/mL lipoproteins of β mobility from patients with familial dysbetalipoproteinemia (type III hyperlipoproteinemia) (Hazzard et al., 1970; Havel & Kane, 1973). For comparison, we also studied subfractions of $d <$ 1.006 g/mL lipoproteins of pre-β mobility from patients with endogenous lipemia.

To determine whether the cholesteryl esters in the cores of these triglyceride-rich lipoproteins exist in any ordered state, we studied the induced CD of β-carotene, a normal constituent of human lipoproteins. In a previous report, we showed that LDL exhibits reversible, temperature-dependent CD spectra attributable to β-carotene between 2 and 37 °C (Chen & Kane, 1974). Further, in a study of reconstituted LDL, we showed that β-carotene is a valid spectroscopic probe for determining the changes in the physical state of core lipids of lipoproteins, since the induced CD of β-carotene accurately reflects thermal transitions of cholesteryl esters placed in the reconstituted lipoproteins (Chen et al., 1980).

Experimental Procedures

Preparation of Lipoproteins. Serum was obtained from eight patients fasted for 14 h. Four had familial dysbetalipoproteinemia (type III hyperlipoproteinemia), and four had endogenous lipemia (Kane et al., 1983). These donors had been given daily dietary supplements of β-carotene, one 30-mg capsule twice daily for 14 days. (The carotene was a gift from Hoffmann-La Roche, Inc.) Lipoproteins of $d < 1.006$ g/mL (VLDL), $1.006 < d < 1.019$ g/mL (IDL), and $1.024 < d <$ 1.050 g/mL (LDL) were isolated by sequential ultracentrifugation in a Beckman L3-50 ultracentrifuge, equipped with a 40.3 rotor (Beckman Instruments, Inc., Palo Alto, CA), at 12 °C for 20 h at 38 000 rpm (Havel et al., 1955). All ultracentrifugal solutions contained 1 mM EDTA, 0.04% NaN_3, and 0.5 mg/mL gentamycin to retard degradation of lipoproteins by hydroperoxidation and by bacteria. After isolation, LDL and IDL were dialyzed overnight at 4 °C against 0.15 M NaCl containing 1 mM EDTA, pH 7.5.

Starch Block Electrophoresis. Lipoproteins of the $d <$ 1.006 g/mL fraction of serum from patients with familial dysbetalipoproteinemia have both β and pre-β mobility upon electrophoresis in an agarose gel. In contrast, lipoproteins of the $d < 1.006$ g/mL fraction from patients with endogenous lipemia or from normal individuals have only pre-β mobility. To separate the β-migrating fraction from the pre-β fraction, the $d < 1.006$ g/mL lipoproteins from patients with familial dysbetalipoproteinemia were subjected to electrophoresis in a starch block (Kane et al., 1983). Serial sections of the starch block were extracted twice with 0.15 M NaCl at 23 °C. The electrophoretic mobilities of these lipoprotein fractions were confirmed by electrophoresis in an agarose gel (Noble, 1968). The fractions with β and pre-β mobility were pooled separately and concentrated in an ultrafiltration cell with the UM-2 membrane (Amicon Corp., Lexington, MA) for gel filtration chromatography.

Gel Filtration Chromatography. To subfractionate the β- and pre-β-migrating fractions of the $d < 1.006$ g/mL lipoproteins, they were chromatographed separately on columns (1 × 100 cm) of 4% agarose (Bio-Gel A-15m, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA) (Sata et al., 1972) after separation by starch block electrophoresis. Columns were preequilibrated with 0.2 M NaCl, 1 mM EDTA, and 0.02% NaN_3, pH 7, at 23 °C. Samples were applied in a volume of 2–3 mL (4–6 mg of protein), and fractions of 1.6 mL were collected at a constant flow rate of 6 mL/h.

Lipoprotein Analysis. The concentration of protein was determined (Lowry et al., 1951) with hydrated bovine serum albumin as a standard. Lipids were extracted from lipoprotein fractions into 30 volumes of chloroform/methanol (2:1 v/v). The contents of cholesterol and cholesteryl ester were determined by an enzymatic procedure (Huang et al., 1975), those of triglycerides by a fluorometric technique (Rush et al., 1970), and those of phospholipids as lipid phosphorus (Stewart & Hendry, 1935). The content of β-carotene was determined spectrophotometrically (Chen & Kane, 1974). After negative staining with potassium phosphotungstate, lipoprotein fractions were examined and photographed with a Siemens 101 electron microscope (Siemens Corp., Medical/Industrial Groups, Iselin, NJ) (Hamilton et al., 1971). Particle diameters were measured on the photographs with a Hipad Digitizer (Houston Instrument), by means of a three-point algorithm. Data were analyzed with a TRS-80 computer (Radio Shack, Fort Worth, TX).

Absorption and CD Spectroscopy. To quantify β-carotene in the lipoproteins, visible absorption spectra were measured with a Cary 15 spectrophotometer at room temperature (Chen & Kane, 1974). To determine the occurrence of a thermal transition, CD spectra of four preparations of each lipoprotein class (each preparation from a different donor) were measured between 7 and 40 °C with a Jasco J-500A spectropolarimeter fitted with a thermostated sample chamber under constant nitrogen flush. The temperature of the chamber was controlled by a circulating water bath and was monitored by a Baily BAT-12 digital thermometer (accurate to ±0.05 °C), which was attached to the chamber. The solution was allowed to equilibrate at each temperature level for 15–30 min. Fused cylindrical silica cells with path lengths of 5–50 mm (Pyrocell S-18-260, Westwood, NJ) were used, depending on the concentration of the lipoproteins, to keep the absorbance of the solution below 2.0. The CD data were expressed in terms of specific ellipticity, $[\psi]$ (in degrees centimeter squared per decagram), on the basis of the concentration of β-carotene. Each CD spectrum was the average of two scans of each sample.

Results

Characterization of Lipoproteins. The elution patterns of gel filtration chromatography of the β-migrating (Figure 1A) and pre-β-migrating (Figure 1B) fractions of $d < 1.006$ g/mL lipoproteins from patients with dysbetalipoproteinemia indicated different distributions of particles. Although in each case an initial peak emerged at the void volume, followed by the gradual appearance of smaller particles, only the β-migrating fraction (Figure 1A) had a clearly resolved second peak. The elution patterns of the $d < 1.006$ g/mL lipoproteins from all four subjects with dysbetalipoproteinemia were similar. The elution patterns of the $d < 1.006$ g/mL lipoproteins from patients with endogenous lipemia resembled those of the pre-β fraction of $d < 1.006$ g/mL lipoproteins from patients with dysbetalipoproteinemia. Optical studies were carried out on subfractions I–V. Samples of each fraction were analyzed for chemical composition and particle diameter.

Electron microscopic examination of LDL, IDL, and gel-filtered subfractions of the $d < 1.006$ g/mL lipoproteins (pooled as in Figure 1) showed spherical particles of different

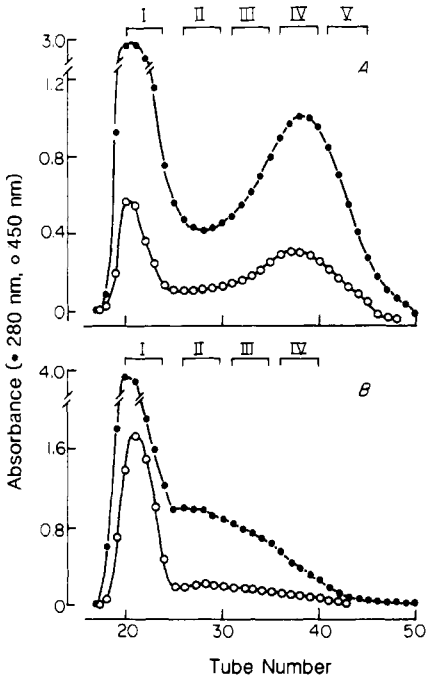

FIGURE 1: Gel filtration patterns [(●) 280 nm; (○) 450 nm, indicating β-carotene content] of the β-migrating (A) and pre-β-migrating (B) fractions of the $d < 1.006$ g/mL lipoproteins from a patient with familial dysbetalipoproteinemia on a β-carotene-enriched diet. The β-migrating and pre-β-migrating fractions, isolated by starch block electrophoresis, were separately applied to a 4% agarose column (1 × 100 cm) at 23 °C. Bars indicate fractions pooled for chemical analysis, particle sizing, and optical measurements.

diameters (Table I). The β-migrating and pre-β-migrating $d < 1.006$ g/mL lipoproteins were heterogeneous, with mean particle diameters ranging from 35 nm for fraction V to 81–85 nm for fraction I (Table I).

All fractions of the β-migrating $d < 1.006$ g/mL lipoproteins from patients with dysbetalipoproteinemia contained more cholesteryl ester and less triglyceride than did the corresponding fractions of the pre-β-migrating $d < 1.006$ g/mL lipoproteins obtained either from patients with dysbetalipoproteinemia (Table I) or from patients with endogenous lipemia. Among the five pooled subfractions of the β-migrating $d < 1.006$ g/mL lipoproteins, fraction I had the highest content of triglyceride. IDL had contents of cholesteryl ester and triglyceride between those of LDL and the β-migrating $d < 1.006$ g/mL lipoproteins (Table I). The composition and particle size data of fractions II and IV of the pre-β-migrating $d < 1.006$ g/mL lipoproteins were not included in Table I because fractions II and IV, like fractions I and III, did not show any induced CD (see below), and the data of fractions I and III were representative of the chemical composition and size of the pre-β-migrating particles.

β-Carotene was found in LDL, in IDL, and in each of the gel-filtered fractions of the $d < 1.006$ g/mL lipoproteins. The contents of β-carotene, expressed in micrograms per milligram of cholesteryl ester, were in the range of 3–4 for LDL and 2–3 for IDL and all the fractions of the $d < 1.006$ g/mL lipoproteins.

Absorption Spectra. All of the lipoproteins studied showed a marked absorbance between 350 and 550 nm, attributable to an increased content of β-carotene, resulting from the carotene-enriched diet (Figure 2). The spectra of the fractions of largest particle diameter were dominated, however, by light scattering (Figure 2, curve 4).

Circular Dichroism. At 7 °C, the pooled, gel-filtered fractions II–V of the β-migrating $d < 1.006$ g/mL lipoproteins

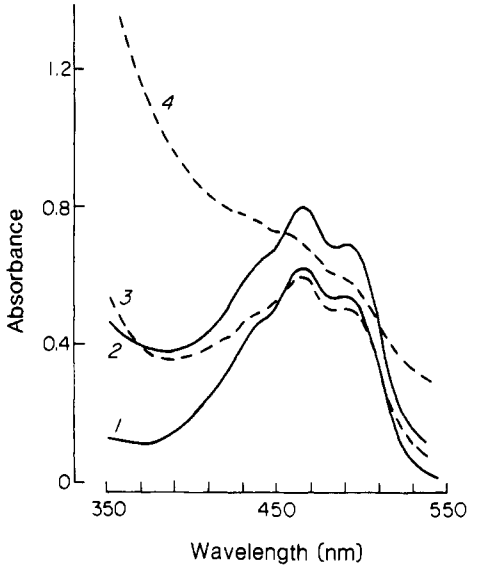

FIGURE 2: Absorption spectra showing the presence of β-carotene at room temperature of LDL (1), IDL (2), and two pooled gel-filtered fractions (3, 4) of β-migrating $d < 1.006$ g/mL lipoproteins from a patient with familial dysbetalipoproteinemia on a β-carotene-enriched diet. Absorbance was measured in a cuvette of 1-cm light path. The concentration of β-carotene (micrograms per milliliter) in samples was as follows: curve 1, LDL (1.9); curve 2, IDL (2.5); curve 3, fraction IV (2.2); curve 4, fraction I (0.9). Fractions I and IV correspond to bars I and IV in Figure 1A.

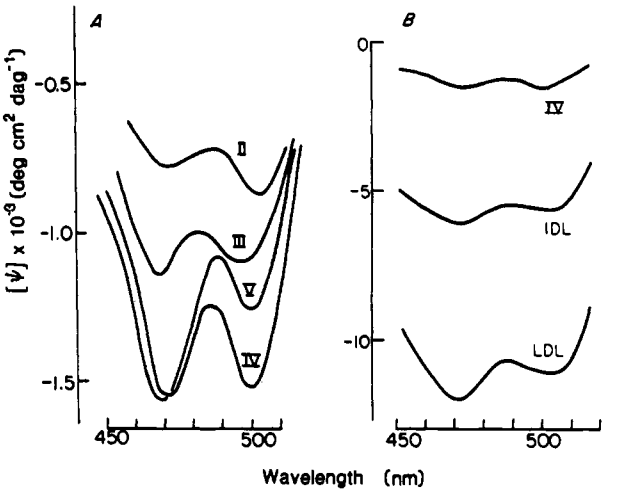

FIGURE 3: CD spectra (based on β-carotene) of various magnitudes at 7 °C of (A) four pooled gel-filtered fractions of β-migrating $d < 1.006$ g/mL lipoproteins and (B) fraction IV, IDL, and LDL from a patient with familial dysbetalipoproteinemia on a β-carotene-enriched diet. Fractions II–V correspond to the bars in Figure 1A.

of patients with familial dysbetalipoproteinemia exhibited CD spectra of different magnitudes of specific ellipticity between 450 and 520 nm (Figure 3A), indicating restraint by ordered lipids upon the β-carotene probe. In contrast, fraction I had no measurable CD in this wavelength region, despite a high content of cholesteryl esters. The CD spectra of fractions IV and V were essentially identical around 450–480 nm. Similar results were observed on three additional preparations from other donors. The positions of the extrema of the CD bands corresponded to the maxima of the absorption bands shown in Figure 2. Similar CD spectra were observed for IDL at 7 °C (Figure 3B). In contrast, none of the pre-β-migrating $d < 1.006$ g/mL lipoproteins obtained either from patients with dysbetalipoproteinemia or from patients with endogenous lipemia showed measurable CD in this wavelength region

Table I: Composition and Size of Lipoproteins from Patients with Familial Dysbetalipoproteinemia

	$d < 1.006$ g/mL lipoproteins								
	pre-β-migrating fractions		β-migrating fractions						
	I	III	I	II	III	IV	V	IDL[a]	LDL[a]
triglyceride[b]	68.5	53.7	51.0	43.6	41.2	36.9	36.2	19.9	7.3
	(3.8)	(2.4)	(3.4)	(5.9)	(6.5)	(9.1)	(6.4)	(1.8)	(2.7)
cholesteryl ester[b]	9.5	13.2	26.6	26.9	23.4	23.8	21.8	33.6	37.9
	(2.8)	(4.0)	(2.9)	(3.6)	(4.0)	(5.7)	(3.8)	(2.0)	(2.9)
cholesterol[b]	5.1	6.2	6.0	7.2	6.9	7.1	6.9	9.7	7.6
	(1.3)	(0.9)	(1.1)	(1.3)	(1.0)	(1.2)	(0.6)	(0.7)	(1.6)
phospholipid[b]	10.7	16.1	13.0	15.9	17.6	19.3	19.1	20.1	21.9
	(3.1)	(2.8)	(3.2)	(1.0)	(1.8)	(1.9)	(1.4)	(2.0)	(2.1)
protein[b]	6.1	10.8	3.4	6.5	11.0	12.9	16.0	16.7	25.4
	(2.1)	(0.8)	(0.5)	(0.4)	(0.5)	(0.5)	(1.0)	(2.8)	(2.6)
TG/CE[c]	7.2	4.1	1.9	1.6	1.8	1.6	1.7	0.6	0.2
particle diameter (nm)[d]	85 ± 21	45 ± 9	81 ± 20	58 ± 10	52 ± 9	41 ± 8	35 ± 6	31 ± 5	22 ± 2

[a] IDL, intermediate-density lipoproteins ($1.006 < d < 1.019$ g/mL); LDL, low-density lipoproteins ($1.024 < d < 1.050$ g/mL). [b] Percent by weight. Values are means (±SD) of three different donors. [c] Mass ratio. TG, triglyceride; CE, cholesteryl ester. [d] Values are means ± SD of a single patient (100 particles were measured in each sample).

under the same conditions, despite a similar content of β-carotene.

Although the contour of the CD spectra of IDL and the β-migrating $d < 1.006$ g/mL lipoproteins resembled that of LDL, the magnitudes of their specific ellipticity were smaller. At 7 °C, the magnitude of the specific ellipticity of IDL was about half to two-thirds that of LDL (Chen & Kane, 1974) but was much larger than that of the subfractions of β-migrating $d < 1.006$ g/mL lipoproteins (Figure 3). The magnitude of these CD bands decreased when the sample was heated (Figure 4). Above 30 °C, the magnitude of all CD bands was very small, and the CD spectra became featureless. This temperature-dependent CD was readily reversible.

Discussion

In this work, we have used induced CD of β-carotene as a spectroscopic probe to study the physical state of the cores of triglyceride-rich lipoproteins. The sensitivity of this technique is demonstrated by the presence of a strong induced CD signal, despite the fact that the content of β-carotene is only 4–6 molecules per VLDL or LDL particle,[2] which contain about 1500 and 1300 cholesteryl ester molecules, respectively (Deckelbaum et al., 1977b). These data show that, as with LDL, the cores of IDL and of the major portion of the β-migrating $d < 1.006$ g/mL lipoproteins exist in some ordered state between 7 and 30 °C, as demonstrated by the induced CD spectra of β-carotene. A previous study of reconstituted lipoproteins has shown that cholesteryl esters are the lipid components responsible for inducing the CD spectra of β-carotene (Chen et al., 1980). Therefore, the observed temperature-dependent CD spectra must reflect the environmental constraint arising from the formation of some ordered state of cholesteryl esters in the cores of IDL and β-migrating VLDL. Changes in the physical state of the cores are also reflected by the temperature dependence of the induced CD spectra (Figure 4).

The existence of the ordered state in the cores appears to be strongly dependent upon the ratio of triglycerides to cholesteryl esters in the lipoproteins. Although the observed, reversible temperature-dependent CD spectra due to β-carotene in IDL and the subfractions of the β-migrating $d < 1.006$ g/mL lipoproteins were strikingly similar in contour to those

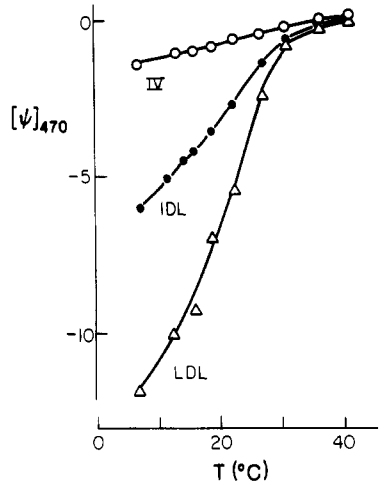

FIGURE 4: Temperature dependence of induced CD of LDL, IDL, and fraction IV. $[\psi]_{470} \times 10^{-3}$ in degrees centimeter squared per decagram.

of LDL (Figure 3B), the magnitude of the specific ellipticity was smaller, and the thermal dependence of the CD band was broader (Figure 4). In addition, although the particle diameter of fractions IV and V of the β-migrating $d < 1.006$ g/mL lipoproteins is similar to the diameter of IDL, their CD behaviors are very different. This progression of differences suggests a disordering effect attributable to triglycerides, an interpretation supported by the progressively greater ratio of triglycerides to cholesteryl esters in IDL (3 times greater) and in the β-migrating $d < 1.006$ g/mL lipoproteins (8 times greater) than in LDL (Table I). This disordering effect of triglycerides is consistent with the effect on the peak temperature and on the magnitude of the thermal transition of cholesteryl esters found by studies of differential scanning calorimetry, and nuclear magnetic resonance (Deckelbaum et al., 1977a; Kroon & Seidenberg, 1982). We have previously observed this effect when triglycerides are incorporated into the cores of reconstituted LDL (Chen et al., 1980).

An additional determinant of the existence of some order in the cores of triglyceride-rich lipoproteins appears to be particle diameter. The gradual increase of mean particle diameter from LDL to IDL and fractions of β-migrating $d < 1.006$ g/mL lipoproteins is accompanied by a decreased magnitude and broader transition of the induced CD bands (Figure 4). Furthermore, in spite of essentially identical ratios

[2] The molecular weight of β-carotene is 537, and that of cholesteryl ester is assumed to be 650.

of triglycerides to cholesteryl esters (Table I) among fractions II–V of the β-migrating $d < 1.006$ g/mL lipoproteins, increasing mean particle diameter from fraction V (about 35 nm) to fraction II (about 58 nm) was associated with progressive decreases in the magnitude of their CD bands (Figure 3A). Finally, fraction I (about 81 nm) showed no measurable induced CD, although its ratio of cholesteryl ester to triglyceride resembled that of the other fractions (Table I). Thus, fraction II of the β-migrating $d < 1.006$ g/mL lipoproteins, with a mass ratio of triglycerides to cholesteryl esters of 1.6, is the largest lipoprotein particle that exhibited induced CD of β-carotene, indicating the existence of some ordered lipids in its cores.

In contrast, fractions of the pre-β-migrating $d < 1.006$ g/mL lipoproteins, obtained either from patients with dysbetalipoproteinemia or from patients with endogenous lipemia, showed no evidence of an ordered lipid domain, even with particle diameters of about 45 nm, as indicated by the absence of detectable CD due to β-carotene, even at 7 °C. The high content of triglyceride (mass ratio of triglycerides to cholesteryl esters >4, Table I) probably accounts for this. This observation is compatible with the absence of a thermal transition in normal human VLDL between 10 and 45 °C by differential scanning calorimetry and by X-ray scattering/diffraction studies (Deckelbaum et al., 1977b).

It has been shown that triglycerides have a profound effect on the physical state of neat cholesteryl esters (Deckelbaum et al., 1977a,b; Kroon, 1981; Small, 1970; Hamilton et al., 1977). Analyses by nuclear magnetic resonance spectroscopy and differential scanning calorimetry in model systems have shown that with 22% triolein present, cholesteryl esters remain liquid until they crystallize (Kroon, 1981; Hamilton et al., 1981). It is remarkable, therefore, that in spite of the high content of triglycerides in IDL (20%) and β-migrating VLDL (36–43%), induced CD of β-carotene allows detection of some ordered state in their cores between 7 and 30 °C. In the study of reconstituted lipoproteins, we have shown that an increasing amount of triolein progressively decreases the magnitude of induced CD of β-carotene at 4 °C (Chen et al., 1980). Only when triolein exceeded 50% of mass was CD undetectable. Thus, the cores of triglyceride-rich lipoproteins can contain some ordered state between 7 and 30 °C, as shown by the induced CD of β-carotene, if they have a relatively low ratio of triglycerides to cholesteryl esters (mass ratio <1.6) and relatively small particle diameters (<60 nm). The ordered lipid in these particles could be small domains in which the structure resembles that observed in LDL, which is detectable only by the exquisite sensitivity of the β-carotene CD probe technique. It is possible that this order is localized to the environment of the β-carotene molecules. Self-association of β-carotene has been described in aqueous dispersions containing phospholipid (Yamamoto & Bangham, 1978). The absence of a CD signal attributable to β-carotene in lipoproteins that are poor in cholesteryl esters, however, suggests that the CD behavior of β-carotene reflects the influence of cholesteryl ester rather than self-association. In any case, it is a natural phenomenon because β-carotene is a normal constituent of triglyceride-rich lipoproteins as well as LDL. It is possible that the order we have detected in β-migrating VLDL is not the same as that detected in LDL by thermal analysis, nuclear magnetic resonance, and carotene probe studies, though it probably involves cholesteryl esters and may be restricted to small domains because its broad thermal dependence suggests less cooperativity. The thermal dependence of the CD of IDL resembles that of LDL more closely, suggesting it represents transitions similar to those of LDL core lipids. In LDL, the cholesteryl ester rich domain is large enough to allow the cooperative melting behavior observed by calorimetry, since differential scanning calorimetry measures the bulk properties of lipids. The induced CD of β-carotene, on the other hand, measures the specific microenvironment in which carotene resides.

Registry No. β-Carotene, 7235-40-7; cholesterol, 57-88-5.

References

Atkinson, D., Deckelbaum, R. J., Small, D. M., & Shipley, G. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1042–1046.

Chen, G. C., & Kane, J. P. (1974) *Biochemistry 13*, 3330–3335.

Chen, G. C., Krieger, M., Kane, J. P., Wu, C.-S. C., Brown, M. S., & Goldstein, J. L. (1980) *Biochemistry 19*, 4706–4712.

Deckelbaum, R. J., Shipley, G. G., Small, D. M., Lees, R. S., & George, P. K. (1975) *Science (Washington, D.C.) 190*, 392–394.

Deckelbaum, R. J., Shipley, G. G., & Small, D. M. (1977a) *J. Biol. Chem. 252*, 744–754.

Deckelbaum, R. J., Tall, A. R., & Small, D. M. (1977b) *J. Lipid Res. 18*, 164–168.

Hamilton, J. A., Oppenheimer, N., & Cordes, E. H. (1977) *J. Biol. Chem. 252*, 8071–8080.

Hamilton, R. L., Havel, R. J., Kane, J. P., Blaurock, A. E., & Sata, T. (1971) *Science (Washington, D.C.) 172*, 475–478.

Havel, R. J., & Kane, J. P. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2015–2019.

Havel, R. J., Eder, H. A., & Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345–1353.

Hazzard, W. R., Lindgren, F. T., & Bierman, E. L. (1970) *Biochim. Biophys. Acta 202*, 517–525.

Huang, H.-S., Kuan, J.-C. W., & Guilbault, G. G. (1975) *Clin. Chem. (Winston-Salem, N.C.) 21*, 1605–1608.

Kane, J. P., Chen, G. C., Hamilton, R. L., Hardman, D. A., Malloy, M. J., & Havel, R. J. (1983) *Arteriosclerosis (Dallas) 3*, 47–56.

Kirchhausen, T., Untracht, S. H., Fless, G. M., & Scanu, A. M. (1979) *Atherosclerosis (Shannon, Irel.) 33*, 59–70.

Kroon, P. A. (1981) *J. Biol. Chem. 256*, 5332–5339.

Kroon, P. A., & Seidenberg, J. (1982) *Biochemistry 21*, 6483–6488.

Laggner, P., Degovics, G., Muller, K. W., Glatter, O., Kratky, O., Kostner, G., & Holasek, A. (1977) *Hoppe-Seyler's Z. Physiol. Chem. 358*, 771–778.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Noble, R. P. (1968) *J. Lipid Res. 9*, 693–700.

Pownall, H. J., Jackson, R. L., Roth, R. I., Gotto, A. M., Patsch, J. R., & Kummerow, F. A. (1980a) *J. Lipid Res. 21*, 1108–1115.

Pownall, H. J., Shepherd, J., Mantulin, W. W., Sklar, L. A., & Gotto, A. M., Jr. (1980b) *Atherosclerosis (Shannon, Irel.) 36*, 299–314.

Rush, R., Leon, L., & Turrell, J. (1970) *Adv. Autom. Anal., Technicon Int. Congr., 1st*, 503–507.

Sata, T., Havel, R. J., & Jones, A. L. (1972) *J. Lipid Res. 13*, 757–768.

Sears, B., Deckelbaum, R. J., Janiak, M. J., Shipley, G. G., & Small, D. M. (1976) *Biochemistry 15*, 4151–4157.

Skipski, V. P. (1972) in *Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism* (Nelson, G. J., Ed.) pp 417–583, Wiley-Interscience, New York.

Small, D. M. (1970) in *Surface Chemistry of Biological Systems* (Blank, M., Ed.) pp 55-83, Plenum Press, New York.
Stewart, C. P., & Hendry, E. B. (1935) *Biochem. J. 29*, 1683-1689.
Tall, A. R. (1980) *Ann. N.Y. Acad. Sci. 348*, 335-351.
Tall, A. R., Atkinson, D., Small, D. M., & Mahley, R. W. (1977a) *J. Biol. Chem. 252*, 7288-7293.
Tall, A. R., Deckelbaum, R. J., Small, D. M., & Shipley, G. G. (1977b) *Biochim. Biophys. Acta 487*, 145-153.
Tall, A. R., Small, D. M., Atkinson, D., & Rudel, L. L. (1978) *J. Clin. Invest. 62*, 1354-1363.
Yamamoto, H. Y., & Bangham, A. D. (1978) *Biochim. Biophys. Acta 507*, 119-127.

Interaction of Apolipoprotein AI from Human Serum High-Density Lipoprotein with Egg Yolk Phosphatidylcholine†

Jacqueline A. Reynolds

ABSTRACT: Apolipoprotein AI from human serum high-density lipoprotein has been recombined with egg yolk lecithin from ternary complexes of detergent–lipid–protein to form homogeneous spherical particles with maximum binding of 220 mol of lipid/2 mol of AI. This complex differs from those formed when *n*-alkyl detergents or short chain saturated diacylphosphatidylcholines interact with AI in that the maximum hydrophobic volume accommodated by the protein is increased as the result of increased α-helix content. Additionally, it is shown that no interaction occurs between AI and didecanoylphosphatidylcholine or egg yolk lecithin above their thermotropic phase transitions and in the absence of single-tail amphiphiles.

Serum lipoproteins are water-soluble complexes of proteins and lipids designed as intravascular transport vehicles for phospholipids, cholesterol, cholesteryl esters, and triglycerides. The major apolipoproteins from high-density lipoprotein (HDL),[1] AI and AII, and from LDL and VLDL, apo B, are characterized by a high degree of "conformational adaptability" in keeping with their physiological function of binding variable amounts of lipid in vivo. AI, which is the subject of this paper, has a molecular weight of 28 400; the primary sequence contains no long regions of solely hydrophobic residues; the predicted secondary structure consists of regions of amphipathic helices; the protein is soluble in aqueous media in the absence of bound amphiphiles. [See Osborne & Brewer (1977) and Scanu et al. (1982) for reviews of the known properties of this protein.] There have been numerous investigations of the amphiphilic binding properties of AI [recent reviews are Reynolds (1982) and Scanu et al. (1982)], and the results can be briefly summarized. (1) *N*-Alkyl detergents bind to a set of three to four independent sites on AI, inducing a conformational change in the protein. (2) The total hydrophobic volume of *n*-alkyl detergents that can be accommodated by one molecule of AI in this conformationally altered state is approximately 4.3×10^4 A^3 and independent of the ligand head group. (3) As first demonstrated by Pownall and co-workers [e.g., see Pownall et al. (1978)] and subsequently repeated by others, association of AI with saturated, bilayer-forming diacyl phospholipids (i.e., those with a minimum alkyl chain length of 14 carbons) is a slow process and highly temperature dependent with the maximum rate of association occurring at the transition temperature of the lipid. Phosphatidylcholines containing unsaturated fatty acyl chains and uncontaminated with single-tail amphiphiles such as fatty acids and lysolecithins have transition temperatures below 0 °C, and these lipids do not complex spontaneously with AI above their transition temperatures (Assmann & Brewer, 1974; Pownall et al., 1981).

Pownall has proposed that interaction of AI with phospholipid bilayers at the thermotropic phase transition in the *absence* of contaminating single-tail amphiphiles occurs by insertion of the protein moiety into regional "defects", a similar mechanism to that proposed for the binding of phospholipase to lipid bilayers (Volwerk & De Haas, 1982). It is significant that a single homogeneous species is not formed when AI is incubated with dimyristoyl- or dipalmitoylphosphatidylcholine at the phase transition temperature, but rather multiple products are observed [e.g., see Jonas et al, (1980) and Swaney (1980)]. Further, reported levels of "saturation" of AI with these phospholipids vary among laboratories. These observations are consistent with previous suggestions that this interaction is a kinetically limited process and the products observed are not necessarily the thermodynamically stable states (Reynolds, 1982; Pownall et al., 1981).

We have previously discussed the kinetic problems inherent in an investigation of the interactions of components one or more of which have limited solubility in the aqueous milieu (Mimms et al., 1981; Watt & Reynolds, 1981; Dhawan & Reynolds, 1983). One experimental approach is to disperse the hydrocarbon-soluble components in detergent-mixed micelles. We have demonstrated that removal of detergent from such multicomponent system of protein–lipid–detergent micelles leads to the formation of the morphological structure

† From the Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710. *Received June 3, 1983.* Supported by U.S. Public Health Service Grant HL30049.

[1] Abbreviations: EYL, egg yolk phosphatidylcholine; SDS, sodium dodecyl sulfate; GdmCl, guanidinium chloride; HPLC, high-performance liquid chromatography; PPOPC, L-α-palmitoyl-2-palmitoleoylphosphatidylcholine; diC_{10}PC, didecanoylphosphatidylcholine; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); VLDL, very low density lipoprotein(s); EDTA, ethylenediaminetetraacetic acid.

0006-2960/84/0423-1124$01.50/0